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This project consisted of three tasks. Results for Tasks 1 and 2 were submitted to the SERDP Project Manager previously as detailed reports. Therefore, the results for these two tasks will only be summarized and drawn from where needed for other sections of this report. For Task 1, we evaluated the potential for advanced *in situ* measurement of ions (e.g., calcium) and water-quality properties (alkalinity, hardness and conductivity) by use of lab-on-a-chip technology, and derived a path to move forward on further development of advanced *in situ* monitoring techniques. At the request of SERDP, funding for this task was provided by Oak Ridge National Laboratory (Offices of Life Sciences and Environmental Technologies, and Partnerships and Program Development). Task 2 included a screening survey of the chemical, physical, and biological characteristics of seven sites in three streams on the Fort Hood Military Reservation near Killeen, Texas. This task served the purpose of identifying a location that could provide a range of environmental conditions that will be needed for "ground-truthing" advanced-monitoring techniques. Task 3 was a detailed literature search for *in situ* bioassay methods that may be suitable for use in developing and validating the use of advanced-monitoring techniques. For this task, special consideration was given to techniques that would be most pertinent to our efforts. The results of Task 3 are given in detail in this report since they have been reported previously.

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December 29, 2000

Prepared for:

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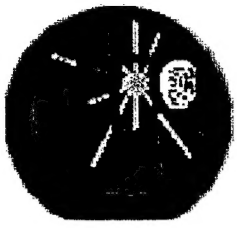

Environmental Sciences Division
Oak Ridge National Laboratory

Management Contractor for DOE's
Oak Ridge National Laboratory

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1.0 INTRODUCTION

This document is our final report for SERDP SEED project (CS-1161), "Feasibility Study: Lab-on-a-chip and *In Situ* Bioassay Techniques for Rapid Resolution of Ion Signatures for Disturbances of Biological Significance in Streams." The work completed under this project addressed the needs of the Department of Defense's (DoD) Strategic Environmental Research and Development Program (SERDP), for Determinants of Indicators of Ecosystem Health (SEEDSON-00-4). The main premise of our project is simple: physical disturbances to the terrestrial environment, such as those that commonly occur at military training facilities (e.g., soil compaction and disturbance, vegetation removal), will generate characteristic "chemical signatures" that are carried into aquatic ecosystems (e.g., streams) in surface runoff. We postulate that by linking and applying two emerging measurement and analysis methods (i.e., "lab-on-a-chip" technology and *in situ* bioassays), the data needed to predict damage and recovery of aquatic systems from terrestrial disturbances can be obtained more rapidly and cost effectively than possible through more conventional methods that are now typically used.

Our project consisted of three tasks. Results for Tasks 1 and 2 were submitted to the SERDP Project Manager previously as detailed reports (Stewart and Smith 2000; Smith and Stewart 2000). Therefore, the results for these two tasks will only be summarized and drawn from where needed for other sections of this report. For Task 1, we evaluated the potential for advanced *in situ* measurement of ions (e.g., calcium) and water-quality properties (alkalinity, hardness and conductivity) by use of lab-on-a-chip technology, and derived a path to move forward on further development of advanced *in situ* monitoring techniques (Stewart and Smith 2000). At the request of SERDP, funding for this task was provided by Oak Ridge National Laboratory (Offices of Life Sciences and Environmental Technologies, and Partnerships and Program Development). Task 2 included a screening survey of the chemical, physical, and biological characteristics of seven sites in three streams on the Fort Hood Military Reservation near Killeen, Texas (Smith and Stewart 2000). This task served the purpose of identifying a location that could provide a range of environmental conditions that will be needed for "ground-truthing" advanced-monitoring techniques. Task 3 was a detailed literature search for *in situ* bioassay methods that may be suitable for use in developing and validating the use of advanced-monitoring techniques. For this task, special consideration was given to techniques that would be most pertinent to our efforts. The results of Task 3 are given in detail in this report since they have not been reported previously.

2.0 ASSESSMENT OF LAB-ON-A-CHIP TECHNOLOGY

2.1 Background

Oak Ridge National Laboratory (ORNL) program development funds were used to conduct Task 1, as requested by the SERDP Program Manager. Task 1 had a single objective: determine the feasibility of using lab-on-a-chip technology (Fig. 1) to develop a chip-sized device capable of providing *in situ* measurements of alkalinity, conductivity and hardness in water at near-real time. We prepared a detailed report on our findings for this task, and submitted it to the SERDP Program Manager in March 2000. How we proceeded with Task 1, and what we learned, is summarized below.

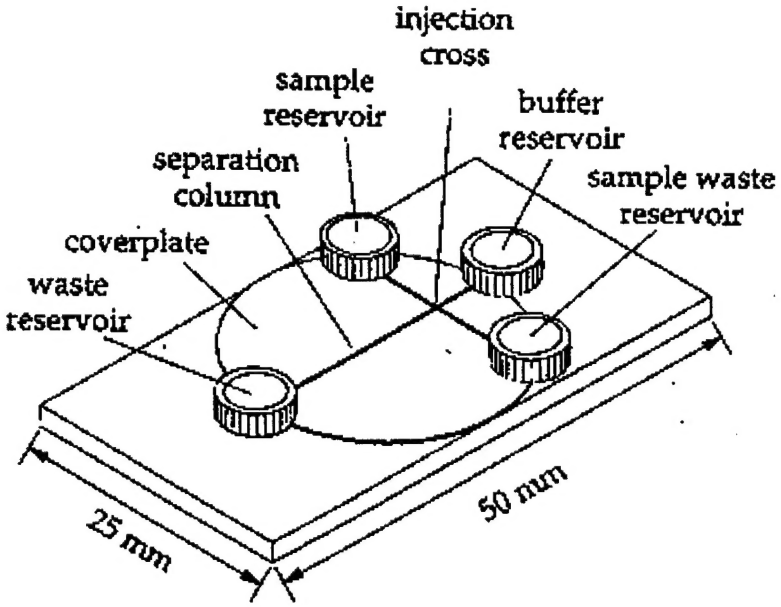

Fig. 1. Schematic of lab-on-a-chip device. (Source: Jacobson et al. 1995)

2.2 Methods

To determine if lab-on-a-chip technology could be used to develop a chip-sized device for measurement of water quality, we queried experts. These included Drs. J. Michael Ramsey (ORNL, Chemical and Analytical Sciences Divison), Thomas Thundat (ORNL, Life Sciences Division), and Stephen Jacobson (ORNL, Chemical and Analytical Sciences Division). Dr. Ramsey is an ORNL Corporate Fellow and a microfluidics expert; his research focuses on microseparation technology and lab-on-a-chip applications. Dr. Thundat, a Senior Scientist, leads the Nanoscale Science and Devices Group. He is the inventor of the microcantilever sensor system, which can be used to quantify constituents that are separated or reacted on a lab-on-a-chip platform. Dr. Jacobson, a Research Scientist in the ORNL Laser Spectroscopy and Microinstrumenation Group, also has much practical experience in development and testing of chip-based chemical analysis techniques. We also spoke with Dr. Kevin Walsh, Associate Professor of Electrical Engineering at the University of Louisville (UL). Dr. Walsh directs the UL's Lutz Microtechnology Cleanroom, and is an expert in microfabrication technology and microelectromechanical systems. Finally, the idea of developing a laser-based technology that could be scaled down to fit a lab-on-a-chip device was discussed with Drs. Meng-Dawn Cheng and Madhavi Martin, in the ORNL's Environmental Sciences Division. Drs. Cheng and Martin have developed laser-induced plasma spectroscopy methods for continuous, multi-elemental monitoring that could be used to detect and quantify metals such as calcium.

2.3 Results

The experts in lab-on-a-chip technology all agreed that microfluidics techniques are available for adding nanoliter or microliter volumes of reagents to water, using computer-controlled, programmed gating to add reagents and establish appropriate reaction zones for the analysis of constituents such as calcium. Thus, chip-based techniques for the colorimetric analysis of alkalinity and hardness are feasible. Conductivity measurements are very simple and can be accomplished by existing microsensors. The Environmental Protection Agency's (EPA) colorimetric methods for analysis of alkalinity and hardness require specific pH adjustments; thus, pH-measuring capabilities would need to be incorporated into the chip-based unit. Dr. Thundat is developing a microcantilever sensor for measuring pH. In the early stages of development of this sensor, Dr. Thundat achieved a linear response to changes in pH over a range of 4 to 10. An issue that would need to be addressed for pH measured by a microcantilever sensor is signal stability and reproducibility, given a flowing-fluid medium. For alkalinity and hardness, a technical consideration is that of detecting and quantifying the colored reaction products. An engineering issue we discussed briefly was that of how to prevent biofouling and clogging of the chip's channels and reaction zones by particles such as detritus, pollen, silt, etc. But several engineering avenues could be used to address this situation: it was viewed as an engineering challenge only.

Hardness and alkalinity analyses (using EPA methods) depend on color-change reactions driven by titration with an appropriate reagent, so quantification by light absorption is possible. This technology is well developed, even for chip-based platforms. Photodiodes that generate light at the desired wavelength can be used, and these light sources are small, stable and relatively long-lived. Fluorometric methods for quantifying calcium are available, but are not suitable for analysis of alkalinity because the fluorescence intensity of most fluorophores is influenced strongly by pH and dissolved metals. Market-ready technology for transporting data streams generated by chip-based analysis devices appears to be available (Hydrolab® representative; personal communication with A. J. Stewart, Oak Ridge National Laboratory).

Laser-induced plasma spectrometry (LIPS) can be used for the analysis of solid-phase calcium (Fig. 2), but because the calcium that contributes to hardness is dissolved, this method probably will not be useful for the calcium measurements needed to estimate hardness. Dr. Martin used LIPS successfully to detect calcium in calcium-carbonate films that were prepared on chitosan-treated fused-silica coupons. Thus, while measurement of dissolved calcium with laser-based technology is not feasible, LIPS could be used in association with methods that measure calcium dissolution and precipitation. This could have potential application for detecting changes in calcium demand, and thus, detecting environmental change.

2.4 Conclusions

We did not encounter any fundamental obstacles to the idea that lab-on-a-chip methods could be used to measure and report on water-quality constituents such as conductivity, alkalinity or hardness. Several of the technologies needed to achieve this objective (notably, data logging and wireless transmission) are already commercially available. Effort would need to be dedicated to sensor selection and chemical approach; effort also would be needed to design the chip. Once the design is complete, the chip could be fabricated either at ORNL or outsourced. Any problems associated with biofouling and clogging of the chip's channels and reaction zones can be handled through engineering avenues. In short, the idea looks like a "go", technologically. If such as chip is designed and constructed, the more difficult task would be that of evaluating its operational reliability, accuracy and durability in the natural environment, and developing a means to suitably collect and analyze the data in a multivariate context.

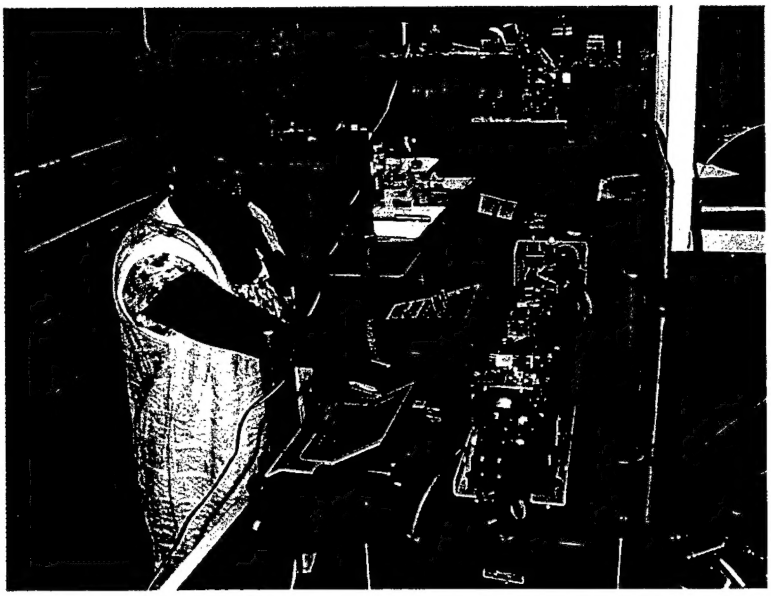

Fig. 2. Laser-induced plasma spectroscopy (LIPS).

3.0 FIELD SURVEY

3.1 Background

Task 2 of our project was a screening survey of the chemical, physical, and biological characteristics of seven sites in three streams on the Fort Hood Military Reservation near Killeen, Texas. Multiple land uses at Fort Hood contribute to physical disturbances of varying magnitudes, making this an excellent location for development of advanced techniques for monitoring disturbances. The physical habitat was characterized at each site using readily available standard procedures, and a chemical survey was conducted of water-quality parameters (1) of direct importance to land-use disturbances, and (2) that could ultimately be measured with lab-on-a-chip devices. The biological survey focused on stream macroinvertebrates and had two objectives: (1) to gain a general idea of the biodiversity supported by these streams, and (2) to identify species that might be suitable for use in *in situ* bioassays during validation studies of the advanced monitoring technique. A secondary objective associated with our field survey was to identify a method for preparing carbonate films, which could potentially be used for monitoring changes in the "demand" of biologically available calcium in streams.

3.2 Methods

In mid-May 2000, seven sites in three streams on the Fort Hood Military Reservation (Cowhouse Creek, Hensen Creek, and Owl Creek) were selected based (1) on a preliminary visit to 13 locations on

eight streams, (2) watershed size and overall hydrological importance to the Reservation, (3) input from staff of the Natural Resources Branch at Fort Hood (Kevin Cagle and John Cornelius), and (4) published information on biota in streams on the reservation (Johnson 1994). The physical habitat of each site was characterized following U.S. EPA's visually-based habitat assessment techniques (Barbour et al. 1999). Conductivity, water temperature, concentration of dissolved oxygen, and pH were measured at each site using portable hand-held meters. Water samples were collected concurrently from each site for determinations of alkalinity, hardness, and a suite of cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, NH_4^{+}) and anions (HCO_3^{-}, SO_4^{2-}, Cl^{-}, PO_4^{3-}, NO_3^{-}, NO_2^{-}); alkalinity and hardness were determined on site, and anions and cations were determined in a laboratory. A single qualitative benthic macroinvertebrate sample was collected from each study site with an aquatic D-frame dip net (500 μm-mesh net). Samples were pre-processed at stream-side and returned to a laboratory for identification to the lowest practical taxon.

Efforts were initiated to develop a technique that could ultimately allow testing of a particular body of water's propensity to dissolve or precipitate calcium. This technique ultimately could provide the ability to obtain information on biologically available calcium by documenting calcium exchanges in water. Preliminary tests were conducted in a laboratory to provide proof-of-principle for the technique. After several unsuccessful attempts to coat a chitosan surface by direct precipitation of calcite, efforts shifted to a method of creating a calcium-carbonate film on chitosan. This approach was successfully accomplished by preparing a slurry of calcium carbonate, allowing the slurry to deposit on a chitosan surface, and drying at 40°C. The durability of the coated films was tested in rapidly flowing water in indoor artificial streams for 3 h. Calcium on the films was measured by laser-induced plasma spectrometry (LIPS).

3.3 Results

Habitat scores for all of the sites at Fort Hood did not differ much, indicating that the overall quality of their habitat was similar (Table 1). Bank erosion, siltation and sedimentation were evident at all study sites, particularly those in close proximity to unpaved roads used for military exercises and where significant areas of vegetation had been cleared. Another important factor contributing to bank erosion was the presence of cattle; these animals are allowed to graze freely on the Fort Hood Military Reservation. While overall habitat scores were similar at the study sites, distinct site-to-site differences also were found. These unique differences were great enough to allow calibration of monitoring techniques to assess on the basis of physical characteristics and habitat diversity.

Water quality results revealed a relatively high degree of accuracy for measuring ions by conventional techniques (ion balances were 88.8% to 98.8% complete). This is important because it shows that repeated measurements of conductivity, alkalinity and hardness, analyzed by simple correlation through time, should be able to provide the desired information even if ion contributions of bioactive materials such as phosphate and nitrate are ignored. Furthermore, total ions measured in a laboratory correlated strongly with conductivity, a coarse-grained surrogate for total ions. We also found that pair-wise correlations among conductivity, alkalinity, and hardness were strong and positive for data pooled across sites (Figs. 3 - 5). Finally, differences in values for conductivity, alkalinity and hardness were large enough to permit ready resolution of site-to-site differences. Thus, data on these parameters should be sufficiently robust to allow the statistical rigor needed for detecting site and time differences using the high collection-frequency data that can be provided by lab-on-a-chip devices.

Initial steps in devising a method that would allow measurements calcium dissolution and precipitation were successful. Chitosan surfaces were successfully coated with calcium-carbonate, and laser-induced plasma spectrometry was used successfully to measure calcium on their surfaces after a brief exposure of the coated surfaces in water (Fig. 6). This is important because it provides a plausible

path forward for the production of calcium-rich films that can be used to track biologically available calcium in aquatic environments.

Table 1. Habitat assessment results using EPA (Barbour et al. 1999) protocols. Values are individual metric scores[a].

	Site[b]						
Habitat Parameter	CC_UP	CC_MI	CC_LO	HC_UP	HC_LO	OC_UP	OC_LO
Epifaunal Substrate/ Available Cover	7	12	8	11	8	11	14
Embeddedness	16	13	12	10	5	4	14
Velocity/Depth Regime	13	12	11	2	9	9	15
Sediment Deposition	14	13	5	8	5	6	5
Channel Flow Status	8	8	6	0	6	7	9
Channel alteration	13	12	14	18	11	14	16
Frequency of Riffles	5	5	6	0	15	10	10
Bank stability							
LB	2	5	4	4	4	9	8
RB	5	4	6	7	7	10	8
Vegetative protection							
LB	1	9	10	10	10	4	10
RB	2	3	5	10	3	5	10
Riparian vegetative zone							
LB	9	5	8	7	10	7	9
RB	7	5	9	4	5	5	9
Total Score	**102**	**106**	**104**	**91**	**98**	**101**	**137**

[a]Maximum scores for each metric are 20, although left (LB) and right (RB) bank scores for Bank stability, Vegetative protection, and Riparian vegetative zone are broken down to maxima of 10 each.

[b]Site names comprise the name of the stream (CC = Cowhouse Creek; HC = Hensen Creek; OC = Owl Creek) and locator (UP = upstream most site; MI = mid-stream site; LO = downstream most site.)

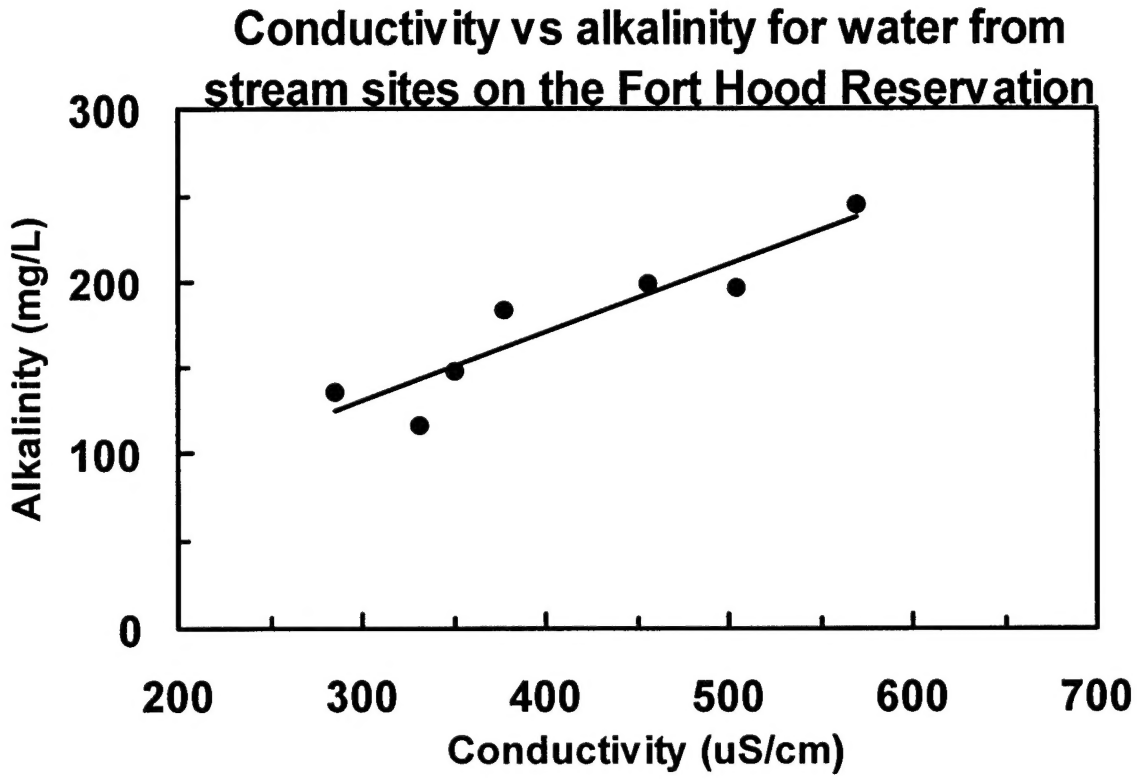

Fig. 3. Relationship of alkalinity versus conductivity for seven stream sites on the Fort Hood Military Reservation.

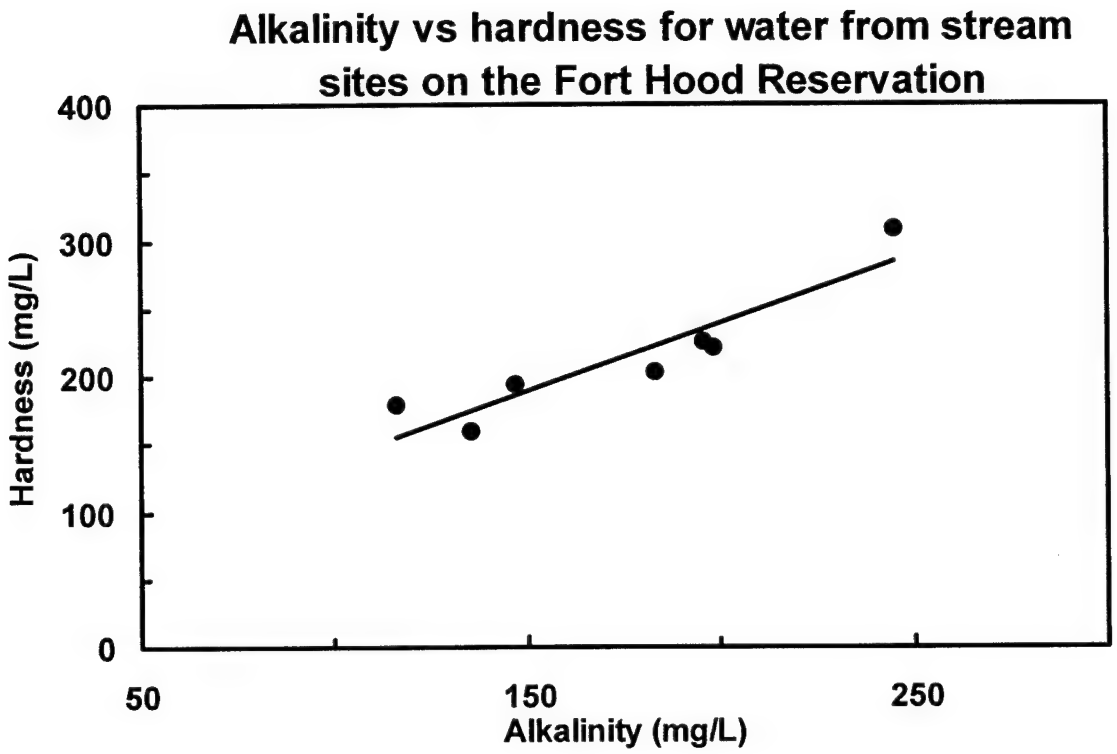

Fig. 4 Relationship of hardness versus alkalinity for seven stream sites on the Fort Hood Military Reservation.

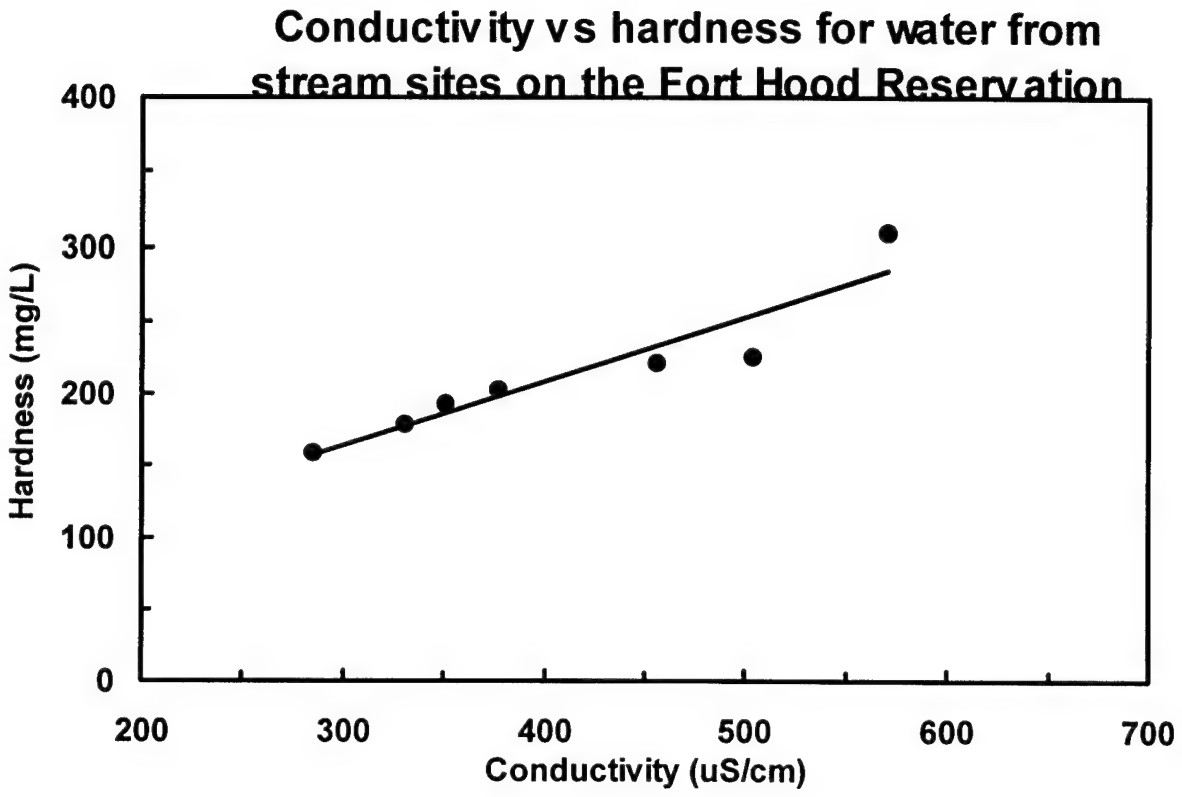

Fig. 5. Relationship of hardness versus conductivity for seven stream sites on the Fort Hood Military Reservation.

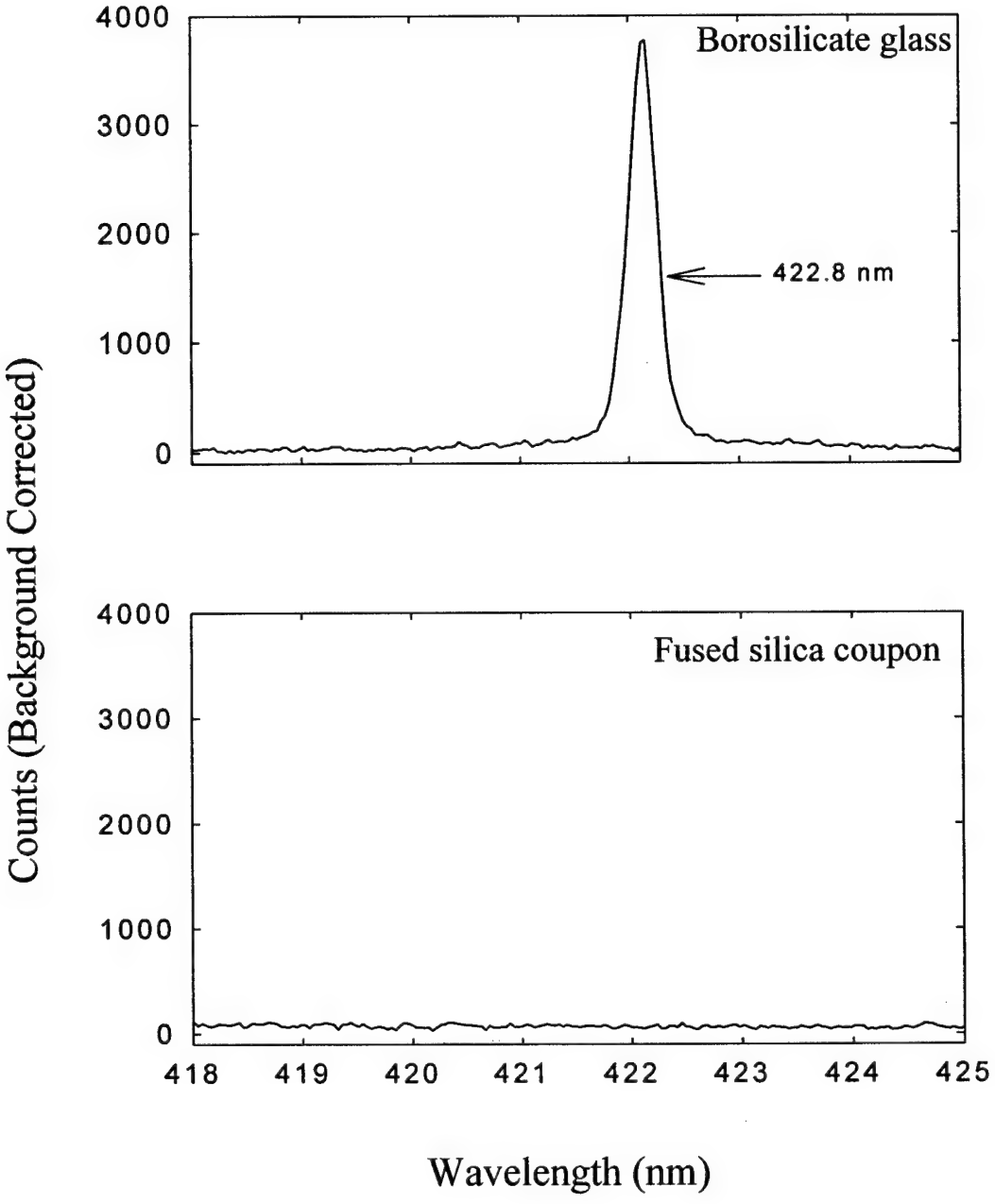

Fig. 6. Analysis of calcium on borosilicateglass slides and fused-silica coupons with laser induced plasma spectroscopy (LIPS).

The screening survey of the macroinvertebrate communities at the seven sites showed that the streams supported a wide variety of taxa, including those often associated with good water quality (e.g., mayflies, stoneflies, and caddisflies; Fig. 7). However, all sites had a general predominance of taxa known to be tolerant to a wide range of environmental conditions. A few taxa were unique to specific sites, but most taxa collected in Hensen Creek and Owl Creek also were present at one or more of the Cowhouse Creek sites. Even with a predominance of relatively tolerant taxa, the differences in the communities were great enough that we were able to detect unique site characteristics from data collected using just a simple screening survey. These differences will be critical for distinguishing effects or changes associated with land-related disturbances from those associated with other man-related or natural factors. Finally, the survey also allowed us to identify several taxa in Fort Hood streams that could be suitable for use in *in situ* bioassays. These included taxa with high calcium requirements (e.g., snails), and taxa that have more "typical" calcium requirements (e.g., many insects) (Brown 1991; Scheuhammer et al. 1997). These differences also are great enough to provide a useful working-range of physiological requirements for calcium.

3.4 Conclusions

The results from Task 2 provided information needed for formulating a path forward to develop, test, and verify an advanced monitoring technique that combines lab-on-a-chip technology with *in situ* bioassays. The land-use practices on the Fort Hood Military Reservation, and the chemical, physical, and biological characteristics of its streams, represent conditions appropriately broad enough for the development and verification of advanced monitoring techniques. This effort could complement and contribute to current efforts in a sediment-and nutrient-transport study on the Fort Hood Military Reservation that is being led by Dr. Dennis Hoffman of the Blackland Research Center in Temple, Texas.

4.0 *IN SITU* BIOASSAYS

4.1 Background

The ultimate goal of our SERDP SEED project was to develop and eventually apply a simple, low cost, time-efficient approach for measuring near real-time changes in water quality that could have ecological significance. Development of any new technique requires validation to ensure that it performs as intended. Bioassays are assays that use some type of biological system (e.g., specific species or groups of species) for measuring a response to a perturbation of interest (Chapman 1995). Thus, bioassays are a logical choice for method validation because they provide information on whether or not a chemical change translates into an important biological response. Bioassays also can provide the desired qualities to link with lab-on-a-chip technology for development of a sound and cost effective monitoring approach: they can be used to provide an early-warning indication of stress at a relatively low cost. Thus, bioassays can serve as one of the tools for detecting changes in environmental conditions (Lagadic and Caquet 1998; Mackay et al. 1989; Smith and Beauchamp 2000).

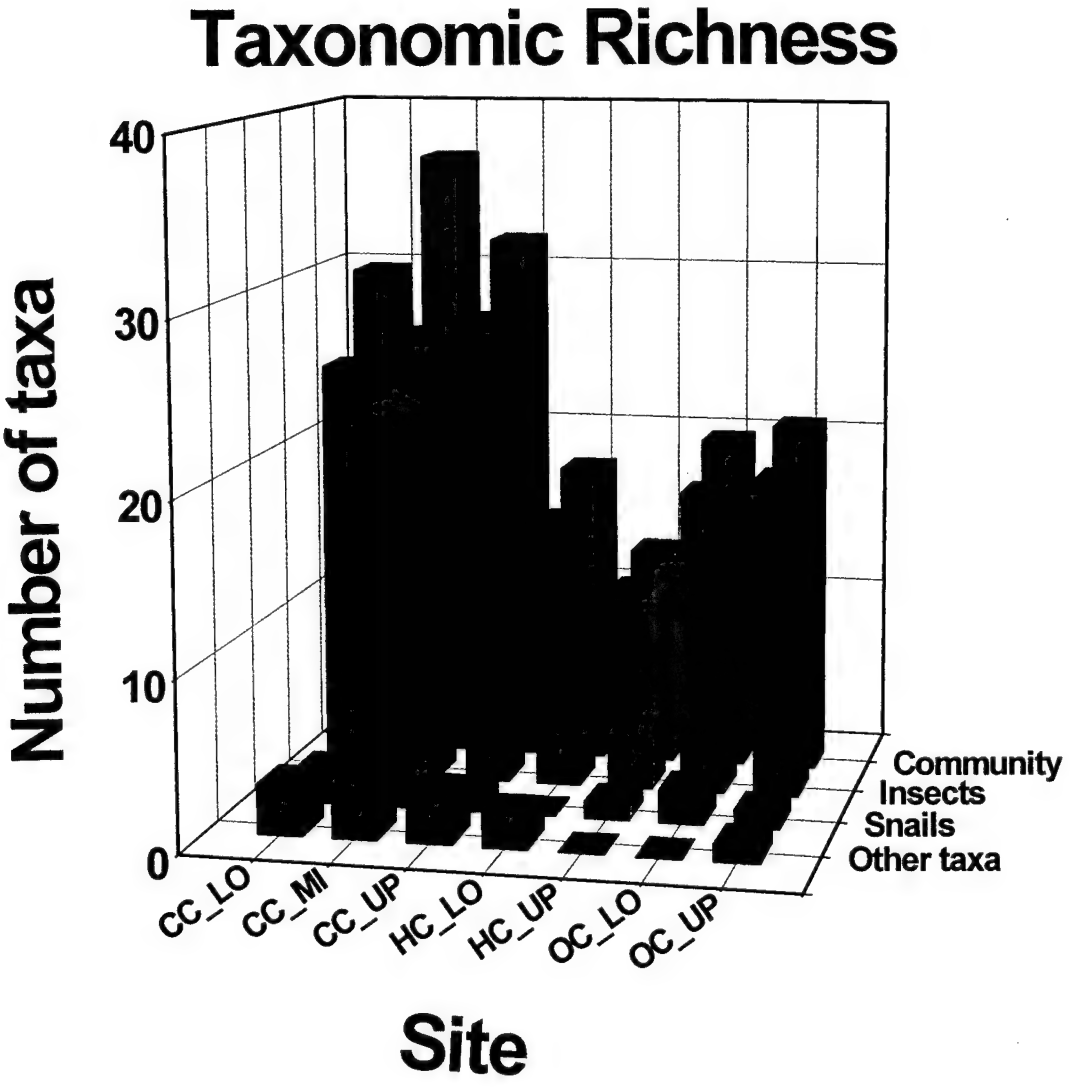

Fig. 7. Taxonomic richness of the benthic macroinvertebrate communities at sampling locations in streams on the Fort Hood Military Reservation, May 10 - 11, 2000.

Chemical tests used alone are not sufficient for predicting environmental damage (APHA 1995; Davis et al. 1996). Typical bioassessment techniques, such as community surveys, can be expensive and time consuming, and although they are generally the most accurate, the information they provide may not be available until well after environmentally important changes have occurred. In short, the time lags are long enough so that if a problem is detected at the community level, it is already too late to implement preventative measures. Bioassays often used in laboratories, such as toxicity tests, cannot provide the realism of exposure conditions that may actually exist (Crane et al. 1995; Seager and Maltby 1989; Schulz and Liess 1997; Turnbull and Bevan 1995). For example, significant episodic changes in important chemical or physical factors can easily be missed in a laboratory since test animals are exposed only to "spot" samples of water from the affected environment. *In situ* bioassays, in contrast, provide continuous exposures to actual water quality conditions (Shaw and Manning 1996). Thus, they can bridge the important gap between less realistic laboratory studies and slower, more expensive field bioassessments (Hopkins 1993; Mackay et al. 1989).

For the third and final task of our SERDP SEED project, we conducted a detailed literature search (but not exhaustive) to identify potential *in situ* bioassay techniques that could be used in streams, such as those at Fort Hood, to (1) validate our proposed approach for predicting environmental damage and recovery, and (2) identify those techniques potentially most suitable for use with our approach. The detailed results of Task 3 follow. While the primary focus of our search was on *in situ* bioassays, we also considered laboratory bioassays and other types of field bioassays that we felt had potential application to our approach.

4.1 Methods

We conducted extensive literature searches on *in situ* bioassay techniques with three commercially available data bases and the North American Benthological Society's data base of their published bibliographies on benthic science (http://www.benthos.org/index.htm). These computer searches were supplemented with pertinent cited references in the articles that were reviewed. Keyword searches included "in situ", "field bioassay", and "situ bioassay." Two sub-areas were searched in the **American Sciences and Fisheries** data base: "Biological Sciences and Living Resources", and "Aquatic Pollution and Environmental Quality." The search in the sub-area, "Aquatic Pollution and Environmental Quality," covered 1990 through September 2000. The sub-area, "Biological Sciences and Living Resources," covered 1978 through September 2000. The search in **Current Contents**, covered September 1994 through August 2000, and the search of the **Science Citation Index** covered 1973 through October 2000. The search of the North American Benthological Society's data base of published bibliographies only includes publications from 1959 – 1992.

4.2 Results

The data base searches yielded 259 "hits", although many of these hits were not unique to one specific database. Table A in Appendix A, summarizes *in situ* bioassay characteristics from pertinent studies, as well as information from other bioassay techniques that may have some application towards advancement of our approach. The latter category included studies that used stream-side channels and channel modifications, or laboratory toxicity tests, microcosms, and/or mesocosms in multidisciplinary studies; studies using fish were not considered unless they were part of a multidisciplinary effort. The focus was on invertebrates because these organisms are less likely than vertebrates to raise concerns from animals rights groups (Lagadic and Caquet 1998). Also, as the size of test organisms increase, bioassays become more expensive and space requirements increase (Cooper and Barmuta, 1993), characteristics not conducive to our intent of developing a rapid and inexpensive approach.

Various types of bioassays have been used in the United States at least since the early 20th century (Mount 1993). The earliest bioassays were conducted in laboratories, and they were generally used to learn about species-specific responses to water quality changes in their natural environment. Emphasis in bioassays in the mid-1900's began shifting more towards toxicity testing in laboratories: toxicity testing of effluents and specific chemicals and compounds. Interest in laboratory toxicity testing reached a peak in the late 1970's and 1980's as it became increasingly evident that measurements or monitoring of chemicals alone could not provide sufficient information to ensure suitable protection of the environment (Burton 1991; Cairns 1989; Hopkins 1993; Persoone and Janssen 1993). Laboratory tests for determining the toxicity of effluents and ambient waters were "formally" standardized in the mid-1980's by the U.S. EPA (Horning and Weber 1985). The importance and impacts of these standardized procedures have been substantial. The proceedings of numerous symposia specifically addressing bioassays have been published in journals since that time, and numerous compilations of articles written by various experts in the application and development of bioassays have been published in books (e.g., Cairns and Niederlehner 1993; Calow 1993; Munawar 1989).

While development and use of laboratory bioassays were major thrusts of environmental evaluations in the 1970's and 1980's, it has only been within that past 10 to 15 years that *in situ* bioassays have begun receiving much attention (Hopkins 1993; Pereira et al. 2000; Sibley et al. 1999). Much of this increased interest developed because of a predominant consensus that laboratory bioassays cannot provide the realism of environmental exposures that can be provided more readily by *in situ* bioassays (Hopkins 1993; Stewart 1996). *In situ* bioassays do not, however, come without criticism. Because conditions of no two streams or stream locations can be duplicated, pseudoreplication can be problematic (Cooper and Barmuta 1993). Understanding this fact about *in situ* bioassays is critical to avoid misinterpretation of results. Cage effects are often mentioned as a potential problem, because cages of any type can alter factors such as food supply or ecological interactions (e.g., Burton 1991; Crowley et al. 1983). While more natural exposures to water quality conditions may be possible with *in situ* bioassays, the containers/cages used to hold organisms can reduce the ecological realism (e.g., affect competition or predation) that could be of potential importance, simply because the test organisms are isolated from a portion of their natural ecological environment (Mackie 1978; Shaw and Manning 1996). This negative trait, however, also can serve as a positive trait since it minimizes the potential confounding influence that these ecological factors can create, thereby allowing one to obtain a more direct response to the surrounding water-quality conditions (Shaw and Manning 1996).

Like laboratory bioassays, the predominant purpose for conducting *in situ* bioassays has been for determining if toxic conditions exist in a body of water or sediments (Appendix A). However, *in situ* bioassays also are commonly used for (1) obtaining biological or ecological information on individuals species, species groups, and communities of organisms (e.g., McMahon and Williams 1986; Crowley et al. 1983), or (2) determining the occurrence and source of contaminants (e.g., Foe and Knight 1987; Mulliss et al. 1996; Peterson et al. 1996). In circumstances where perturbations may have significantly impacted streams at their origins, *in situ* bioassays may serve as useful screening tools for identifying early improvements in water quality. Community responses can be delayed under such circumstances because of the loss of the potential colonizing organisms from upstream sources (Smith and Beauchamp 2000). The problem associated with loss of a major source of colonizing organisms can be further exacerbated if the changes occur during a period when major recruitment does not occurr, such as late fall or winter.

Unlike laboratory bioassays, standard procedures for *in situ* bioassays do not exist (APHA 1995; Stewart 1996). Stewart (1996) suggested that standardization of *in situ* bioassays is unlikely to occur for some time because the need for laboratory-derived toxicity data in risk assessments and biomonitoring programs is outpacing development and acceptance of new techniques for *in situ* testing by the scientific community. Most *in situ* aquatic bioassays use a single species, and expose the test animals at the

desired location in some type of cage or chamber for varying periods of time (Appendix A). Cages can be designed to allow exposures in the water column or sediments, and to isolate individuals or groups of individuals. Common materials used for cages include bags made from mesh small enough that test animals cannot escape, or short pieces of opaque or white PVC or plastic tubing that has side or end windows covered with a fine-mesh net to allow the surrounding water to mix in the cages. Other types of *in situ* bioassays include mark and recapture studies in which individuals animals are captured, uniquely marked, and released at a study site. In reciprocal or transfer bioassays, the organisms of choice are allowed to colonize some type of artificial substrate at a reference location over a period of several days or weeks (e.g., bacteria and algae), and then transferred to other study sites for a brief period of time before being retrieved for analyses.

In addition to *in situ* bioassays, stream-side experiments that use water diverted from the study stream can be used to improve environmental realism (Appendix A). Artificial channels (e.g., guttering, modified pipes) or tanks (e.g., aquaria) placed adjacent to the body of water of interest can be supplied with water from a study site that is delivered via gravity feed or pumped into a head tank before being released into the channels or tanks (e.g., Cooper and Barmuta 1993; Ham and Peterson 1994; Poirier and Surgeoner 1988; Rempel and Carter 1986). This method allows direct exposures to actual water quality conditions for a test site, and provides potential experimental options not available with *in situ* bioassays, such as temperature control, removal or reduction of particulates, or if desired, the addition of a specific chemical or chemical compound of interest. A related but less-used technique involves dividing a stream channel into two or more separate channels with some type of a partition (e.g., Leland et al. 1989), or dosing of an entire stream segment with a toxicant or other manipulation of interest (e.g., Stewart 1987; Wallace et al. 1986, neither in Appendix A). Studies such as these can also be performed in standing bodies of water by setting up barriers between two or more selected areas of the water body (Schindler and Fee 1974, not in Appendix A). While stream-side studies and studies that separate areas with partitions can increase environmental realism, they generally require more materials and effort, which increase costs.

Microcosms and mesocosms provide more realistic simulations of natural environmental conditions similar to those in actual field settings (Appendix A; Cooper and Barmuta 1993; Shaw and Manning 1996). These methods are generally associated with conditions designed to mimic standing waters. Microcosms and mesocosms are usually colonized with an assortment of species collected from existing bodies of water. Microcosms can be set up in a laboratory or in the field; the larger size of mesocosms though limits them to use in outdoor settings. *In situ* bioassays can also be incorporated into microcosms or mesocosms. This strategy allows the investigator to evaluate the direct effects of water quality without the potential confounding effects of other ecological influences, such as predation or competition.

Many groups of test animals have been used in *in situ* bioassays including protozoa, algae, invertebrates, and fish (Appendix A). Often, the test animal(s) end(s) up either being indigenous to the waters being tested, or having a very wide distribution (Pontasch et al. 1989). However, species typically used in laboratory bioassays have been used successfully in *in situ* bioassays as well (e.g., Chappie and Burton 1997; Periera et al. 2000). But no one species can serve as a surrogate for the response of an entire biological community in a body of water, so attempts should be made to use more than one species, and if possible, consider more than one life stage (Cairns 1983; Cairns and Cherry 1993; Crane et al. 1995; Lagadic and Caquet 1998; Parkhurst 1993). Invertebrates tend to be used much more frequently than vertebrates for several reasons. First, invertebrates generally have attributes that make them particularly useful test animals, such as high abundance, ease of and tolerance to handling and being reared under controlled conditions, and in many cases, wide distribution ranges. They also span the range of ecological rolls in aquatic ecosystems (herbivores, detritivores, predators, etc.) (Lagadic and Caquet 1998). These characteristics provide the opportunities needed to select species based on specific

study needs and desired endpoints. For example, the importance of calcium could be studied by using test animals that might have high calcium needs (e.g., mollusks), in combination with specific developmental stages or processes of another species that might have lower but more specific, narrow calcium requirements (e.g, possibly eggs or species that molt frequently) (Brown 1991; McMahon 1991; Edmunds and Waltz 1996).

Numerous endpoints have been used in *in situ* bioassays, but identification and selection of the most sensitive and informative endpoints to meet specific needs creates the greatest challenge for addressing the biological and ecological importance of abnormal changes in ion balances (Appendix A). Survival is the most commonly used endpoint in *in situ* bioassays, but reproduction and particularly growth (which provide information of sub-lethal toxicity) are used almost as frequently (Mount 1993). Responses such as survival and growth generally correlate well with actual biological conditions only when environmental conditions are fairly toxic: as conditions improve, these metrics become less sensitive (Parkhurst 1993). Other endpoints that have been used successfully for detecting sublethal conditions include natality, behavior changes (e.g., valve movement of bivalves, movement within their environment), scope for growth, ecological changes (e.g., density or taxa richness when the test incorporates species groups or some level of community representation), feeding rate, insect emergence, condition index, and biomarkers such as physiological or biochemical changes (Appendix A). Bioaccumulation is used frequently, but only for contaminant source identification, or simply to determine the presence or absence of contaminants and their quantities. Measurements of physiological or biochemical changes of many species, and valve movement of bivalves, are endpoints that may hold the most promise for obtaining sensitive and rapid responses to change. However, it might be possible to identify two or more species with narrow and specific requirements for ions that could be used to measure a sublethal endpoint (e.g., loss of calcium from mollusks).

4.3 Summary

A variety of bioassay techniques exist, particularly *in situ* techniques, that can be used or modified to provide method validation and incorporate into an approach that has predictive capabilities; final design will be determined by specific study objectives and the types of test animals available. Suitable test animals can be obtained for *in situ* bioassays from either indigenous populations, or from laboratory cultures of species typically used in laboratory toxicity tests. Stream-side bioassays, in which source-water can be diverted or pumped into or through artificial channels or tanks, have potential as well. Such techniques could provide more experimental control than *in situ* bioassays, but the increased sophistication and material needs of these types of bioassays could substantially increase costs and time commitments. Thus, they may not serve the intended goal of developing a "faster and cheaper" method for predicting ecological damage or recovery.

While *in situ* bioassays can be used to obtain rapid predictive results at low cost, studies dealing with environmental damage have clearly demonstrated that the greatest degree of accuracy is obtained by use of multi-tiered or multidisciplinary studies that incorporate a combination of laboratory and field bioassays with field bioassessments (e.g., Cherry et al. 2001; Crane et al. 1995; Poirier and Surgeoner 1988; Pontasch et al. 1989; Soucek et al. 2000). Extensive studies clearly would be needed (and justified) for validating and further developing of our predictive approach. Both laboratory and field bioassays (*in situ* and possibly stream-side) also would be needed to test hypotheses on the relationships among our suite of conservative water quality properties (i.e., conductivity, alkalinity, and hardness), calcium, and biological responses. Furthermore, tests are needed to distinguish between the physical effects of sediment or silt, and those resulting from fluctuations in concentrations of ions such as calcium. A final step in the validation process would include a field bioassessment of invertebrate or periphyton communities from sites in streams where nearby terrestrial disturbances, sedimentation and

siltation, and ion concentrations are known. This battery of tests would help validate our approach, and provide the best means for identifying bioassays most suitable for use in conjunction with lab-on-a-chip monitoring devices.

The greatest challenge for using *in situ* bioassays is the selection of sensitive and reliable endpoints for determining biologically significant responses to changes in ions (e.g., calcium). Many of the endpoints commonly used in bioassays lack the sensitivity needed to detect subtle yet important biological changes (e.g, mortality, growth, reproduction). Physiological or biochemical changes in test animals may be well suited for detecting rapid responses to environmental change, but behavioral endpoints (e.g., valve movement of bivalves) also could be suitable. We expect to be able to identify other sensitive endpoints by more closely considering test animals or specific life stages of test animals that have unusually high or narrow calcium requirements.

5.0 PROJECT INTEGRATION

In our SERDP SEED project we have accomplished an in-depth examination of three distinct areas that are critical for the successful development and application of a state-of-the-art approach for predicting ecological damage and recovery: (1) evaluate the feasibility of using lab-on-a-chip devices to obtain near real-time water quality measurements; (2) survey a range of streams at a military base where terrestrial disturbances caused by military training activities create a broad range of stream impacts; and (3) identify candidate *in situ* bioassay techniques that could be incorporated into our approach. To be effective, the approach should be able to provide an inexpensive, rapid method to measure near real-time changes in the water quality of streams that could be biologically significant. This method could be used as an early warning device (i.e., predictive capability) to detect changes in water quality of potential ecological significance. Similarly, the approach could be used to predict the potential for ecological recovery before coarser-scaled measurements of biological changes (and many ecological processes) can be detected.

Based on discussions with experts at ORNL and in academia, we think it will be possible to develop lab-on-a-chip devices that can collect near real-time data on important yet simple water quality characteristics (i.e., conductivity, alkalinity, and hardness). A great challenge in the development of lab-on-a-chip will be the evaluation of its reliability, accuracy, and durability when used *in situ*, and development of a means to suitably collect and analyze the data in a multivariate context. Many options exist for development of a suitable *in situ* bioassay that could be used in conjunction with a lab-on-a-chip device. Suitable test animals should be available from indigenous populations, but if not, test animals that have wide-spread distributions or that are commonly used in standardized laboratory toxicity tests are available. Another big challenge for our approach will be the identification of test endpoints for use in *in situ* bioassays that will be sensitive enough to detect subtle but potentially ecologically relevant changes in ions; behavioral, physiological or biochemical changes in test animals may be among the most suitable for detecting rapid changes. Verification of our approach would require a multi-tiered testing approach that includes laboratory bioassays, and field bioassays (e.g., *in situ* and/or stream-side) and bioassessments of several streams that provide a broad range of watershed disturbances and water quality characteristics. Surveys of streams on the Fort Hood Military Reservation showed that suitable chemical, physical, and biological conditions exist there to eventually field test our approach.

6.0 FUTURE DIRECTION

In a full-scale study, we will (1) begin designing a chip-based analytical system capable of performing on-board analyses of conductivity, alkalinity and hardness; (2) investigate biological

responses to short-time-scale fluctuations in semi-conservative water-quality properties; and (3) develop a statistically sound process for analyzing the relationships between conductivity, alkalinity and hardness (see Stewart 2000). We are confident that we can make excellent progress on these three tasks over a three-year period.

6.1 Recommended tasks

Task 1 (design of chip-based analytical system) will involve experienced staff at ORNL, the University of Louisville, and a representative from the private sector (e.g., Hydrolab®). We think that for this task, the most productive path forward is that of hiring a postdoctoral fellow, and assigning 100% of that individual's time to the final design of a lab-on-a-chip device. This individual would spend some of his or her time working with Dr. Walsh at the University of Louisville, and Drs. Thundat, Ramsey and Jacobson at ORNL. A portion of the funding for this task would need to be allocated to Drs. Walsh, Ramsey, Thundat and Jacobson for their guiding roles. We expect most of the design criteria to be completed within two years, and will plan for a prototype analyzer to be available near the end of the third year. This task could be divided into discrete activities that can be monitored closely to ensure an adequate overall rate of progress.

Task 2 will focus on biological responses of stream-dwelling organisms to short-time-scale fluctuations in semi-conservative water-quality properties. This task will use aquatic organisms identified from our work on the Fort Hood Military Reservation, and will test these organisms for their sensitivity to deviations in concentrations of alkalinity-producing salts (e.g., sodium bicarbonate), hardness constituents (e.g., calcium and magnesium), and materials such as sodium sulfate (which contribute to conductivity, but which do not affect hardness or alkalinity). The experiments to determine the biological significance of short-term fluctuations in conductivity, alkalinity and hardness will be conducted in a set of indoor artificial streams available as a unique facility at ORNL (Fig. 7; Steinman et al. 1991; http//www.esd.ornl.gov/facilities/index.html). This stream system includes eight, long U-shaped channels, and have been used previously for stream ecosystem studies funded both by the National Science Foundation and the Department of Energy.

For **Task 3**, a statistically sound procedure for extracting information from conductivity, alkalinity and hardness time-series data will be constructed. The objective is to develop criteria that can be used to determine quantitative tolerance criteria, so that one can determine when changes have occurred with a specified level of statistical confidence. The method that has been used to date involves use of a associational procedure (see Stewart 2000); this procedure is not particularly efficient at extracting information inherent in time-series data, and thus, does not take best advantage of the high-frequency flux of information afforded by a lab-on-a-chip device. Experts in time-series analysis are available at ORNL and other locations; we will work with these individuals to complete this task.

6.2 Partners

We expect to work with Dr. Walsh (University of Lousiville) on Task 1, due to his expertise in microfabrication, and we will identify one or more DoD and private sector partner(s) on this task, as well. On Task 2, we expect to retain close communication with John Cornelius and Kevin Cagle, at the Fort Hood Military Reservation, and with Dr. Dennis Hoffman (Texas A&M Blackland Research Center). Dr. Hoffman directs stream-monitoring studies on the Fort Hood Military Reservation, which focuses on the export of sediment from the Reservation. For Task 3, we will draw from expertise at ORNL and a collaborator from academia.

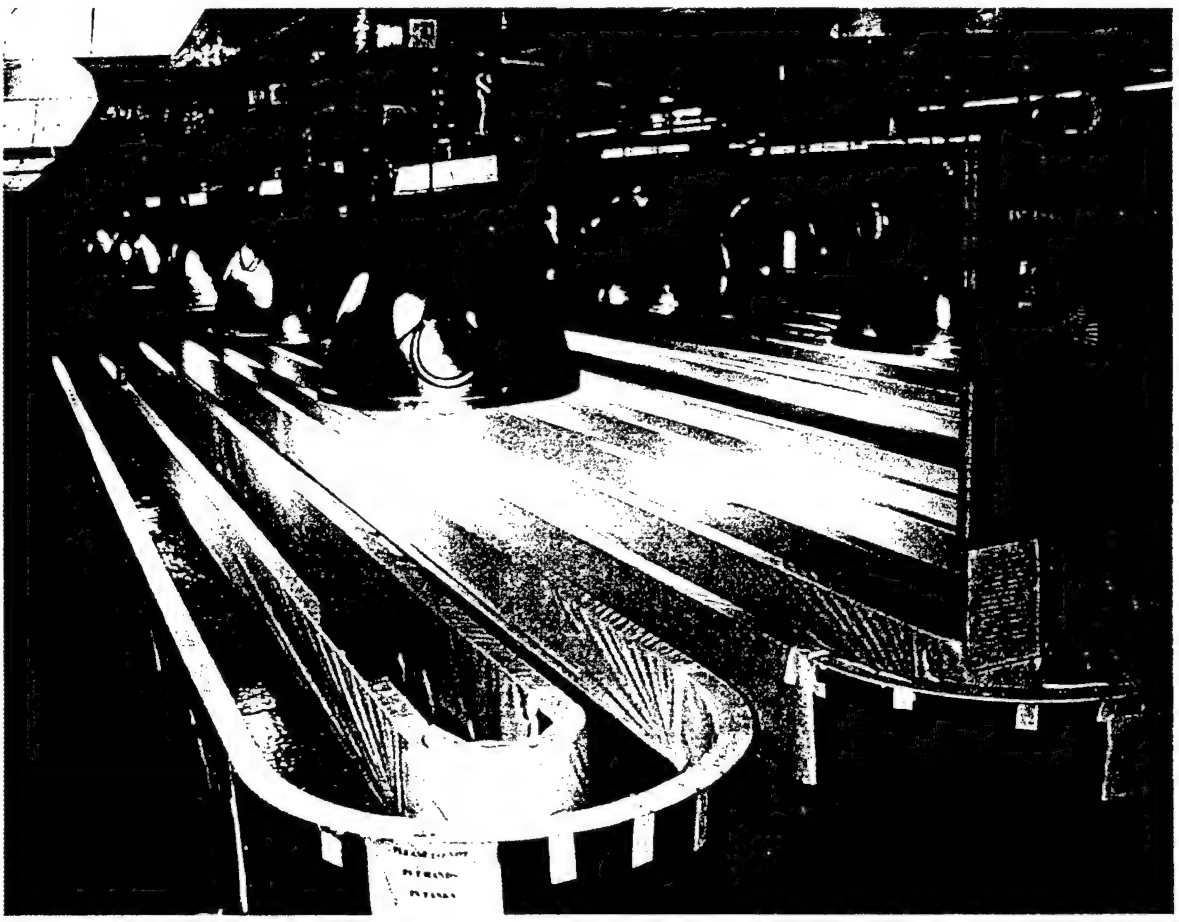

Fig. 7. Artificial streams in the Aquatic Ecology Laboratory at Oak Ridge National Laboratory.

6.3 Preliminary budget estimate

The recommended tasks described above are expected to require approximately $ 450 to $ 500 K per year. A breakdown of projected annual budgets by task is given in Table 2.

Table 2. Estimated budget for recommended tasks to complete development and validation of an advanced approach for predicting damage and recovery from environmental perturbations.

Task	Year 1	Year 2	Year 3	Total
Design of chip-based analytical system (1)	$250K	$200K	$100K	$550K
Biological responses (2)	$200K	$250K	$250K	$700K
Data-stream analysis (3)	–	$50K	$150K	$200K
Totals	$450K	$500K	$500K	$1,450K

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APPENDIX A

Summary of *In Situ* Bioassays

Table A. Summary of representative *in situ* bioassays and bioassays potentially pertinent to development of advanced monitoring approaches. (References are given in Section 7.0, Literature Cited)

Study	Bioassay	Test apparatus	Test purpose(s)	Test organism(s)	Endpoint(s)	Exposure time
Allred and Giesy 1988	Microcosm	Mesh bags in floating trough	Nutrient effects on leaf breakdown	Sweet gum leaves Water oak leaves`	Mass loss, chemical composition	30 wk
Burris et al. 1990	*In situ*	Mark and recapture	Method development, water quality	*Elimia clavaeformis*	Movement	24-h and 48-h trials
	Laboratory streams	Mark and recapture	Chlorine toxicity	*Elimia clavaeformis*	Movement	2 h/d for 8 d
Chappie and Burton 1997	*In situ*	Clear-capped tubes w/mesh windows	Method development	*Hyalella azteca* *Chironomus tentans*	Survival	2 d - 4 wk
Cherry et al. 2001[a]	*In situ*	Mesh bags	Water quality monitoring, toxicity	*Corbicula fluminea*	Mortality	35 d
Crane et al. 1995[a]	*In situ*	PVC tubes capped w/mesh	Water quality assessment	*Gammarus pulex*	Mortality, feeding rate	28 d
	In situ	Wire mesh cages	Water quality assessment	*Anodonta cygnaea*	Mortality, glutathione-S-transferase induction	28 d
	Laboratory	Stream sediment in beaker	Sediment toxicity	*Chironomus riparius*	Emergence	28 d
Crane and Maltby 1991	*In situ*	PVC tubes capped w/mesh	Method development, water quality assessment	*Gammarus pulex*	Mortality, feeding rate	6 d

Table 1. Continued

Study	Bioassay	Test apparatus	Test purpose(s)	Test organism(s)	Endpoint(s)	Exposure time
Crowley et al. 1983	*In situ*	Cylinder of chicken wire covered with fine mesh, capped with plastic dishes	Methods development, competition	Macroinvertebrate community	Density	≈1 mo
Doherty et al. 1987	Laboratory	Artificial streams	Toxicant effects on bioaccumulation	*Corbicula fluminea*	Valve movement, bioaccumulation	≤24 h
Foe and Knight 1986	*In situ*	Plastic cages of egg-crate-type panelling	Method development, water quality monitoring	*Corbicula fluminea*	Mortality, growth, reproduction, condition index, bioaccumulation	10 mo
Foe and Knight 1986	*In situ*	Plastic cages of egg-crate-type panelling	Temperature effects assessment	*Corbicula fluminea*	Mortality, growth, scope for growth condition index, bioaccumulation	6.5 mo
Ham and Peterson 1994	Stream-side	Stream water pumped into tanks	Toxicant effect on behavior	*Corbicula fluminea*	Electronically measured valve movement	24 h
Ireland et al. 1996	*In situ*	Clear capped plastic tubes w/mesh windows	Effects of UV-B on acute toxicity	*Ceriodaphnia dubia*	Survival	48 h
	Laboratory	Beakers	Effects of UV-B on chronic toxicity	*Ceriodaphnia dubia*	Survival	7 d

Table 1. Continued

Study	Bioassay	Test apparatus	Test purpose(s)	Test organism(s)	Endpoint(s)	Exposure time
Kramer et al. 1989	Laboratory	Flow through tanks	Method development, chlorine toxicity	*Dreissena polymorpha* *Mytilus edulis*	Electronically measured valve movement	24 h
Leland et al. 1989	Instream	Stream channel divided by partition	Toxicity	Invertebrate community	Drift, abundance, diversity, 2° production	≈4 mo
	Stream-side	Mesh-covered glass chambers in stream-side channels	Toxicity	Selected Ephemeroptera	Survival	7 - 9 d
Mackie 1978	Laboratory	Sediment in Pyrex dishes	Method development, toxicity, water quality assessment	*Musculium securis*	Natality	Until 75% of adults dead
	In situ	Capped plastic chambers w/mesh windows	Method development, toxicity, water quality assessment	*Musculium securis*	Natality	Until 75% of adults dead
Maltby 1992	Laboratory	PVC tubes capped with mesh and placed in artificial streams	Method development, toxicity	*Gammarus pulex*	Scope for growth, feeding rate, no-observed-effect concentration	28 d
Maltby and Crane 1994	*In situ*	PVC tubes capped with mesh	Method development, water quality monitoring	*Gammarus pulex*	Feeding rate, bioaccumulation	6 d

Table 1. Continued

Study	Bioassay	Test apparatus	Test purpose(s)	Test organism(s)	Endpoint(s)	Exposure time
Maltby and Crane 1994	Laboratory	PVC tubes capped with mesh placed in 15-L glass tanks	Method development, toxicity	*Gammarus pulex*	Mortality, feeding rate, bioaccumulation	6 d
Maltby et al. 1990a	Laboratory	Unspecified type of chamber	Stress effects (toxicants and DO)	*Gammarus pulex*	Scope for growth	6 d
Maltby et al. 1990b	*In situ*	PVC tubes capped with mesh	Method development, water quality monitoring	*Gammarus pulex*	Scope for growth, energy absorbed and respired	6 d
Matthiessen et al. 1995	*In situ*	PVC tubes capped with mesh	Water quality assessment	*Gammarus pulex*	Feeding rates, survival	11 wk
	Laboratory	Nylon mesh pots in aquaria	Toxicity	*Gammarus pulex*	Mortality, feeding rate	7 d
McMahon et al. 1986	*In situ*	Cage made of mesh galvanized hardware cloth	Autecology	*Corbicula fluminea*	Life history, growth rate	≈1 y
	In situ	Mark and recapture	Autecology	*Corbicula fluminea*	Life history, growth rate	≈1 y
Mulliss et al. 1996	*In situ*	Plastic containers covered with mesh	Water quality assessment/monitoring	*Gammarus pulex*	Mortality, bioaccumulation	36 d
Napolitano et al. 1994	Transfer	Periphyton-colonized tiles transferred from reference to study site	Water quality monitoring	Periphyton community	Chlorophyll a, photosysthesis, fatty acids	3 - 35 d

Table 1. Continued

Study	Bioassay	Test apparatus	Test purpose(s)	Test organism(s)	Endpoint(s)	Exposure time
Naylor et al. 1989	Laboratory	Unspecified type of chamber	Method development	*Gammarus pulex*	Scope for growth, energy absorbed and respired	6 d
Ormerod et al. 1987[a]	*In situ*	200-ml plastic container covered with mesh and held within a perforated 2nd container	Toxicity, pH effects	*Chironomus riparius, Hydropsyche angustipennis, Dinocras cephalotes, Ecdyonurus venosus, Baetis rhodani,* and *Gammarus pulex*	Mortality	2 - 3 d
	In situ	PVC pipe (35 cm long) capped with mesh	Toxicity, pH effects	*Salmo trutta, Salmo salar*	Mortality	2 - 3 d
Pereira et al. 2000	*In situ*	Closed polypropylene beakers w/3 mesh windows	Water quality monitoring, toxicity, method development	*Daphnia magna*	Survival, fertility, days to reproduction, growth	10 - 26 d
	Laboratory	EPA toxicity protocols	Water and sediment toxicity	*Daphnia magna, Ceriodaphnia dubia*	Survival, fertility, days to reproduction, growth	10 - 26 d
Peterson et al. 1994	*In situ*	Polypropylene mesh cages	Bioaccumulation	*Corbicula fluminea*	Contaminant accumulation	4 wk

Table 1. Continued

Study	Bioassay	Test apparatus	Test purpose(s)	Test organism(s)	Endpoint(s)	Exposure time
Peterson et al. 1994	Stream-side	Stream water pumped into tanks	Toxicant effects on contaminant uptake	*Corbicula fluminea*	Electronically measured valve movement, growth, bioaccumulation	4 wk
Peterson et al. 1996[b]	*In situ*	Cylindrical polypropylene cages	Bioaccumulation	*Rhinichthys atratulus*	Contaminant accumulation	12 wk
Poirier and Surgeoner 1988[c]	Stream-side	Vinyl channels gravity-fed with stream water	Insecticide toxicity	*Simulium, Isonychia, Pycnopsyche, Acroneuria, Ophiogomphus, Orconectes*	Drift	1 h
	In situ	Plexiglass cages covered with nylon mesh	Toxicity	*Simulium, Isonychia, Pycnopsyche, Acroneuria, Ophiogomphus, Orconectes*	Mortality	24-h pre-through 24-h post-exposures
Pontasch et al. 1989	Laboratory	Standard EPA acute and chronic toxicity test protocols	Effluent toxicity	*Daphnia magna, Ceriodaphnia dubia, Pimephales promelas*	Survival, reproduction	24 h and 7 d
	Laboratory	Microcosm (artificial streams)	Effluent toxicity	Invertebrate and protozoan communities	Various communities metrics	20 d, invertebrates; 7 and 21 d, protozoa

Table 1. Continued

Study	Bioassay	Test apparatus	Test purpose(s)	Test organism(s)	Endpoint(s)	Exposure time
Pontasch et al. 1989	Transfer	Colonized polyurethane foam artificial substrates	Water quality assessment	Protozoan community	Various community metrics	7 d
Rempel and Carter 1986	Stream-side	Mesh bags in PVC channels gravity-fed with stream water	Thermal effects	Green maple leaves	Leaf decay rate, insect colonization	2 - 6 wk
	Stream-side	Mesh-bags in PVC channels gravity-fed with stream water	Thermal effects	Periphyton community	Periphyton photosynthesis and respiration	14 d, 28 d, and 93 d
	Laboratory	Maple leaves colonized in aquaria with microbes from stream water	Thermal effects	Microbial community	Respiration, photosynthesis	2 wk
Salazar and Salazar 1997	*In situ*	Undescribed cages	Water quality monitoring	*Mytilus galloprovincialis*	Growth, bioaccumulation	84 d
Sasson-Brickson and Burton 1991	Laboratory	EPA procedures for 48-hr acute toxicity	Sediment toxicity	*Ceriodaphnia dubia*	Survival	24 h and 48 h
	In situ	Acrylic chambers with mesh bottom for sediment exposure and inlet/outlet tubes on cap for water exchange	Sediment toxicity	*Ceriodaphnia dubia*	Survival	48 h

Table 1. Continued

Study	Bioassay	Test apparatus	Test purpose(s)	Test organism(s)	Endpoint(s)	Exposure time
Schulz and Liess 1997	*In situ*	Floating boxes two-thirds submersed containing sand and with end walls covered with 1-mm mesh	Water quality monitoring, pesticide toxicity	*Limnephilus lunatus, Gammarus pulex*	Survival	82 d
Shaw and Manning 1996[d]	*In situ* exposures in outdoor pond microcosms	Suspended tube covered with mesh	Water column toxicity	*Notonecta, Buenoa*	Growth, survival	72 h
	In situ exposures in outdoor pond microcosms	Polyethylene "pot" capped with mesh placed on pond sediment	Sediment toxicity	*Cuenis, Hyalella azteca*	Growth, survival	10 d
Sibley et al. 1999	*In situ*	Plastic core tube with mesh on bottom and water-exchange tubes on top; pushed into sediment ≈⅓ of length	Method development, toxicity	*Chironomus tentans, Lumbriculus variegatus*	Survival, growth	10 d
Smith and Beauchamp 2000	*In situ*	Opaque PVC cylinders capped with mesh	Water quality monitoring, method development	*Sphaerium fabale*	Growth, survival, natality	70 - 135 d

Table 1. Continued

Study	Bioassay	Test apparatus	Test purpose(s)	Test organism(s)	Endpoint(s)	Exposure time
Soucek et al. 2000[a]	*In situ*	Mesh bags	Toxicity	*Corbicula fluminea*	Survival	31 d
	Laboratory	EPA procedures	Toxicity	*Ceriodaphnia dubia*	Survival	48 h
	Laboratory	EPA procedures	Sediment toxicity	*Daphnia magna*, *Chironomus tentans*	Survival and reprodution (*Daphnia*); survival and growth (*Chironomus*)	10 d
Stewart et al. 1992	Laboratory	Contaminated and uncontaminated macrophytes in white trays holding dechlorinated tap water	Effect of contaminants on food preference	Amphipoda, *Elimia*	Movement	2 d and 7 d, Amphipoda; 48 h, *Elimia*
	Laboratory	Contaminated and uncontaminated macrophytes in beakers of dechlorinated tap water	Effect of contaminants on food preference	*Elimia*	Movement	48 h
	Laboratory	Glass bowls of water with contaminated or uncontaminated macrophytes	Contaminant effect on food quality	*Elimia*	Growth	9 wk

Table 1. Continued

Study	Bioassay	Test apparatus	Test purpose(s)	Test organism(s)	Endpoint(s)	Exposure time
Stewart et al. 1992	*In situ*	Bunches of contaminated and uncontaminated macrophytes in a stream	Food preferences of indigenous snails	Indigenous populations of *Elimia*	Colonization of snails on macrophyte bunches	24 h
	Laboratory	EPA toxicity testing procedures on contaminated and uncontaminated macrophyte leachates	Acute and chronic toxicity	*Ceriodaphnia dubia*	Survival, fecundity	24 h (acute) 7 d (chronic)
Turnbull and Bevan 1995[a]	*In situ*	PVC cylinders capped with mesh	Toxicity	*Gammarus pulex*	Survival	≈145 d
van den Brink et al. 1996	Lentic outdoor	Mesocosm	Toxicity	Macroinvertebrate and zooplankton communities	Various community metrics	55 wk
Warren et al. 1995	*In situ*	Glass cylinders capped with mesh	Water quality monitoring	*Utterbackia imbecillis*	Survival	7 d
Wiackowski et al. 1994	*In situ*	Cubical containers	Predation, competition	*Diacyclops bicuspidatus thomasi, Daphnia rosea, Diaptomus novamexicanus, Holopedium gibberum*	Abundance	8 d

[a]Also included benthic macroinvertebrate community sampling.
[b]Resident species also monitored for bioaccumulation.
[c]Instream invertebrate drift also collected.
[d]Also included benthic macroinvertebrate community sampling in microcosms.

Table 1. Continued

Study	Bioassay	Test apparatus	Test purpose(s)	Test organism(s)	Endpoint(s)	Exposure time
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	Laboratory	EPA toxicity testing procedures on contaminated and uncontaminated macrophyte leachates	Acute and chronic toxicity	*Ceriodaphnia dubia*	Survival, fecundity	24 h (acute) 7 d (chronic)
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Warren et al. 1995	*In situ*	Glass cylinders capped with mesh	Water quality monitoring	*Utterbackia imbecillis*	Survival	7 d
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